ORIGINAL ARTICLE

Effect of energy sources and levels supplementation on ruminal fermentation and microbial protein synthesis in dairy steers

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Abstract

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Four rumen fistulated dairy steers were randomly assigned according to a 4 x 4 Latin square design with a 2 x 2 factorial arrangement of dietary treatments (Factor A = source of energy; CM = corn meal, CC = cassava chip), (Factor B = level of supplementation; 1 and 2% of BW). Four dietary treatments were used: CM1, CM2, CC1 and CC2, respectively. The results exhibited the differences between levels of CM and CC as energy sources. Supplementation of cassava chip at 2 %BW resulted in lower ruminal pH did of CM1, CM2, CC1 (P<0.05). However, ruminal VFA concentrations were similar. Moreover, cellulolytic bacteria populations decreased according to energy source and supplementation level. Furthermore, DM and CP digestibilities were significantly different (P<0.05) among treatments. The result from this experiment suggested that energy sources; cassava chip, affected bacteria and fungi ecology (P<0.05), especially at high level of supplementation at (2% BW).

Key words : cassava chip, corn meal, rumen fermentation

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ผลของแหล่งพลังงานและระดับที่เสริมต่อกระบวนการหมักของกระเพาะรูเมน และกระบวนการสังเคราะห์จุลินทรีย์โปรตีนในโคนมเพศผู้

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ทำการสุ่มโคนมเพศผู้เจาะกระเพาะจำนวน 4 ตัวให้ได้รับอาหารทรีทเมนต์ตามการจัดแบบ 2 x 2 แฟคตอเรียลในแผนการทดลองแบบลาตินสแควร์ (4 x 4) โดยมีปัจจัยแหล่งอาหารพลังงาน 2 แหล่ง คือ ข้าวโพดบด (CM) และมันเส้น (CC) และระดับที่เสริม 2 ระดับคือ 1 และ 2 % ของน้ำหนักตัว ผลการทดลองแสดงให้เห็นว่าแหล่งพลังงานและระดับที่เสริมมีผลต่อกระบวนการหมักในกระเพาะรูเมนแตกต่างกัน โดยเฉพาะอย่างยิ่งโคที่ได้รับการเสริมมันเส้น 2 % ของน้ำหนักตัวมีความเป็นกรดในกระเพาะรูเมนสูงสุด อย่างไรก็ตาม ความเข้มข้นของกรดไขมันที่ระเหยได้ในกระเพาะหมักไม่แตกต่างกัน ประชากรจุลินทรีย์กลุ่มแบคทีเรียที่ย่อยสลายเยื่อใยในกระเพาะหมักมีความแตกต่างกัน ทั้งนี้เนื่องจากแหล่งพลังงานและระดับการเสริมที่แตกต่างกัน นอกจากนี้ความสามารถของการย่อยได้ของโภชนะได้แก่วัตถุแห้งและโปรตีนมีค่าแตกต่างกันทางสถิติ ผลจากการศึกษาวิจัยครั้งนี้ชี้ให้เห็นว่าแหล่งหลังงานและระดับที่เสริมต่างกันมีผลต่อนิเวศวิทยาของแบคทีเรียและเชื้อรา (P<0.05) ในกระเพาะหมักรูเมนแตกต่างกันโดยเฉพาะอย่างยิ่งเมื่อโคได้รับการเสริมแหล่งพลังงานจากมันเส้นที่ระดับ 2 % ของน้ำหนักตัว

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Cassava (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropical regions of Africa, Asia and Latin America. It thrives in sandy-loam soils with low organic matter, and climate characterized by low rainfall and high temperature (Wanapat, 2000). Cassava tubers contain high levels of energy and minimal levels of crude protein and have been used as readily fermentable energy in ruminant rations (Wanapat, 2003). Cassava contains 650-850 g of total non-fiber carbohydrates (TNFC) per kg dry matter (DM) and has been used extensively as a feed for livestock (Kanjanaputhipong *et al*., 2001). Cassava compared to cereal such as maize grain has been reported to have a relatively low crude protein, accompanied by a high rate and extent of degradation in the rumen (Holzer *et al*., 1997). Cassava has a similar digestibility value to steam-flaked corn, but higher than sorghum grain. However, the responses to cassava chip, which is highly degradable in the rumen compared with corn meal, as energy sources have not been extensively studied in dairy steers when fed with urea-treated rice straw. Therefore, this study was conducted to compare the effects of energy sources and supplementation level on ruminal fermentation, microbial protein synthesis and digestibility in dairy steers.

Materials and Methods

Animals, diets and experimental design

Four fistulated dairy steers (Holstein Friesian based, 180±10 kg initial BW) were randomly assigned according to a 2 x 2 factorial arrangement in a 4 x 4 Latin square design to compare the effects of energy sources and supplementation levels with urea-treated rice straw (UTS) on ruminal fermentation, nitrogen balance, feed intake and digestibility of nutrients as well as ruminal microbial protein synthesis. The dietary treatments were as follows: T1 = supplementation of corn at 1 % BW (CM1); T2 = supplementation of corn at 2 %BW (CM2); T3 = supplementation

of cassava chip at 1 %BW (CC1); T4 = supplementation of cassava chip at 2 %BW (CC2).

All animals received cottonseed meal at 0.5 kg/head/day and urea-treated rice straw (5%) was offered *ad libitum* as a roughage source. All animals were kept in individual pens and received free choice of water. The experiment was conducted for four periods, each period lasted 21 days. During the first 14 days, all animals were fed on respective diets, while during the last 7 days, the animals were kept in metabolism crates for total feed collection during which they were restricted to 90% of the previous voluntary feed intake.

Data collection, sampling procedures and analysis

Feeds were randomly collected and fecal samples were taken from total collection of individual cow during the last 7 days of each period. They were analyzed for chemical compositions (DM, CP, ash, NDF, ADF) (AOAC, 1985; Georing and Van Soest, 1970).

At the end of each period, rumen fluid and jugular blood samples were collected at 0, 1, 2, 4, 6 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by using a 60-ml hand syringe at each time. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer). Rumen fluid samples were then filtered through four layers of cheesecloth. The samples were divided into three portions. The first portion was used for NH_3-N analysis where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 x *g* for 15 minutes and supernatant was stored at -20ºC prior to NH3-N and VFA analyses using a HPLC (Instruments by controller water model 600E; water model 484 UV detector; column Novapak C_{18}; column size 4 mm x 150 mm; mobile phase 10 mM H_2PO_4 (pH2.5)) according to Samuel *et al*. (1997). The second portion was fixed with 10% formalin solution in normal saline (0.9% NaCl, Galyean, 1989). Total direct count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco). The third portion was taken to study cultured groups of viable bacteria using roll-tube technique (Hungate, 1969) for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

Samples of blood (about 10 ml) were drawn from the jugular vein at the same time as rumen fluid sampling and was separated by centrifugation at 500 x *g* for 10 minutes and stored at -20ºC until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (HPLC), as described by Chen *et al*. (1993).

The amount of microbial purines absorbed (X mmol/day) corresponding to the purine derivatives excreted (Y mmol/day) was calculated based on the relationship derived by Chen and Gomes (1995).

$$Y = 0.85X+(0.385W^{0.75})$$

where Y is the excretion of purine derivatives (mmol/day); X is the microbial purines absorbed (mmol/day).

The supply of microbial N in gram per day was estimated as follows:

Microbial N (gram/day) =

$$\frac{X \times 70}{0.116 \times 0.83 \times 1000} = 0.727 \times X,$$

with X being the absorption of purine derivatives in mmol/day, following the assumptions made by Chen and Gomes (1995).

*Digestibility of microbial purine is 0.83, the N content of purines is 70 mg N/mmol and the ratio of purine-N : total N in mixed rumen microbes is 11.6 : 100.

The EMNS which denote the microbial N supplied to the animal per unit of DOMR was calculated using the following formula:

$$EMNS = \frac{MN(g/day) \times 1000\ (g)}{DOMR\ (g)}$$

Where DOMR = DOMI x 0.65 (ARC, 1990), DOMR = digestible organic matter apparently fermented in the rumen, DOMI = digestible organic matter intake, EMNS = efficiency microbial nitrogen synthesis, OMDR = organic matter truly digested in the rumen.

Statistical analysis

The means of each parameter measured in the digestibility studies and nutrient intake were analyzed by the analysis of variance (ANOVA) techniques using the general linear model (GLM) procedures of the (SAS, 1998). Treatment means were compared by the least significant difference method (LSD). The 2 x 2 factorial analysis of variance was used to examine the effects of energy source and the level of supplementation as well as their interactions. Rumen fluid data (pH, ammonia-N and VFA) were analyzed by split-plot analysis of variance (Snedecor and Cochran, 1967) using the following model: $Y_{ijklm} = \mu + A_i + P_j + T_k + e_{ijk} + H_i + (AH)_{il} + (PH)_{jl} + (TH)_{kl} + e_{ijklm}$ while where μ is the mean of A, P, T and H which stand for animal, period, treatment and time effects, respectively e_{ijk} is the main plot error and e_{ijklm} is the sub-plot error.

Mean separations with a significant *F* ($P < 0.05$) for treatment were statistically compared using the Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

Results

Composition of the diets

Values of nutrient compositions on DM basis of corn meal, cassava chip, cottonseed meal and urea-treated rice straw are shown in Table 1.

Effect on rumen fermentation and feed intake

The daily DM intake and nutrient digestibility patterns are presented in Table 2. DM intake of UTS and concentrate were affected ($P<0.05$) by energy sources and levels of supplementation. Furthermore, apparent digestibility of DM, CP and ADF were significantly influenced by energy sources and levels of supplementation while OM and NDF were similar in all groups.

Rumen parameters to temperature, pH, ammonia-nitrogen (NH_3-N) and blood urea nitrogen (BUN) concentrations were measured. As shown in Table 3, rumen temperatures were similar among treatments and the values were quite stable at 39ºC. Ruminal pH values measured immediately very 30 minutes from 1-6 h-post feeding were found in a range of 5.2-6.7 which were significantly different among group. As shown in Figure 1, the supplementation of CC2 resulted in lowest pH, while the supplementation at CM1 and CC1 were similar.

Ruminal NH_3-N and BUN concentrations were significantly different ($P<0.05$) with the different energy sources i.e. higher in CM diets. The increases in rumen NH_3-N levels resulted in increasing levels of BUN and the values linearly increased were in CM diets. This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis (Table 2). The effect of both energy sources and levels of supplementation were apparent in both acetate and

Table 1. Chemical compositions (% DM) of cassava chip (CC), corn meal (CM), cottonseed meal (CSM), and urea-treated rice straw 5% (UTS) in the experiment.

Item	CC	CM	CSM	UTS
DM	90.1	89.2	93.7	53.6
OM	96.2	98.3	93.7	87.0
Ash	3.7	1.7	6.2	13.0
CP	2.9	10.1	41.9	8.7
NDF	7.0	15.8	28.0	72.0
ADF	6.2	10.6	21.4	55.0

Table 2. Influence of different energy levels on feed intake and digestibility of nutrients in dairy steers.

Item	Treatment [d]				Contrast [e]			
	CM1	CM2	CC1	CC2	SEM	ES	L	ES x L
DM intake (kg/hd/df)								
UTS	2.7[a]	2.0[ab]	2.0[ab]	1.5[b]	0.21	*	*	NS
Conc.	1.6[a]	3.1[b]	1.7[a]	2.6[b]	0.20	NS	**	NS
Total	4.4[ab]	5.2[a]	3.7b	4.2[ab]	0.33	*	NS	NS
Apparent total-tract digestibility (%)								
DM	69.6[a]	78.5[b]	77.1[b]	82.2[b]	2.37	*	*	NS
OM	70.4	79.0	79.9	79.8	2.88	NS	NS	NS
CP	75.1[a]	74.6[ab]	72.2[c]	72.9[bc]	0.93	*	NS	NS
NDF	62.5	64.7	64.0	61.5	1.28	NS	NS	NS
ADF	59.5[a]	56.3[b]	59.2[a]	55.6[b]	0.82	NS	*	NS

[abc]Values in the same row with different superscripts differ (p<0.05).
[d] CM1 = Corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW.
[e] Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = P<0.05, ** = P<0.01, NS = P>0.05.
[f] UTS = Urea-treated rice straw, Conc. = concentrate.

Table 3. Influence of different energy levels on rumen ecology and fermentation characteristic in dairy steers.

Item	Treatment [d]				Contrast [e]			
	CM1	CM2	CC1	CC2	SEM	ES	L	ES x L
Ruminal Temperature (^oC)	39.8	39.8	39.7	39.8	0.11	NS	NS	NS
Ruminal pH	6.5[a]	6.3[b]	6.5[a]	5.3[c]	0.65	NS	**	NS
NH_3-N (mg/100ml)	7.3[a]	7.3[a]	5.1[b]	5.8[b]	0.55	*	NS	NS
BUN (mg/100ml)	2.9[a]	2.3[ab]	1.4[b]	1.3[b]	0.38	*	NS	NS
Total VFA (mmol/l)	81.1	85.9	85.9	89.4	3.55	NS	NS	NS
Molar proportion of VFA (mol/100mol)								
Acetate (C2)	73.0[a]	70.3[b]	72.6[a]	69.4[c]	0.71	*	**	NS
Propionate (C3)	16.8[a]	19.8[b]	17.0[a]	19.9[b]	0.24	NS	**	NS
Butyrate (C4)	10.1	9.9	10.4	10.4	0.37	NS	NS	NS
C2:C3 ratio	4.3[a]	3.5[b]	4.2[a]	3.4[b]	0.68	NS	**	NS
C2+C4 : C3 ratio	4.9[a]	4.0[b]	4.8[a]	4.0[b]	0.75	NS	**	NS

[abc]Values in the same row with different superscripts differ (p<0.05).
[d] CM1 = Corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW.
[e] Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = P<0.05, ** = P<0.01, NS = P>0.05.

propionate molar proportions. Molar proportion of acetate was significantly higher (P<0.05) in dairy steers fed CM1 (73.0%) than in CC2 (69.4%). In addition, the propionate was also affected (P<0.01) by the level of supplementation when fed by CC being higher when supplemented with at 2%BW

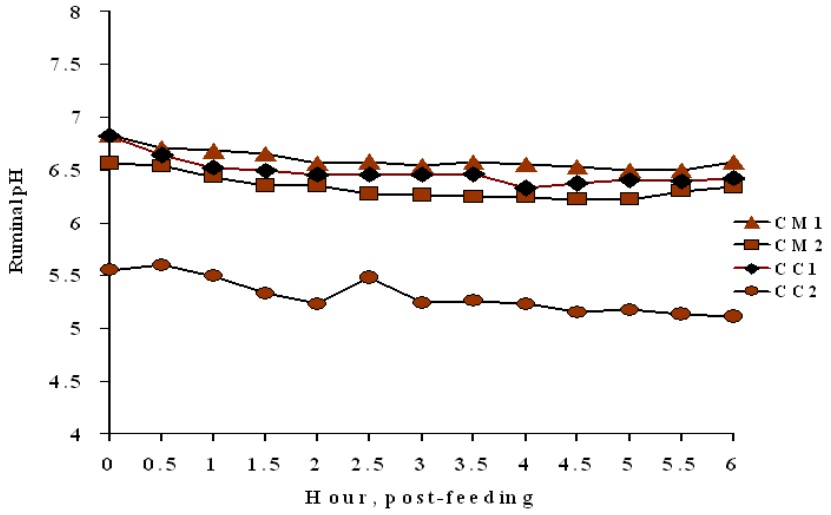

Figure 1. Effect of levels of energy feed supplementation (corn meal at 1% BW, CM1; corn meal at 2% BW, CM2; cassava chip at 1% BW, CC1; cassava chip at 2% BW, CC2) on ruminal pH in dairy steers.

(19.9%) than with at 1%BW (17.0%). As a result, the acetate to propionate (C2:C3) ratio differed between supplementation levels. In terms of CM diets, CM2 was significantly lower C2:C3 ratio (3.5) than those fed CM1 (4.3) and in CC diets, while in CC2 there was a significantly lower ratio (3.4) than in those fed CC1 (4.2). Molar proportion of butyrate tended (P>0.05) to be higher in CC diets (average of 10.4%) than in CM diets.

Effect on rumen microbes

Table 4 presents rumen microorganism population. As for group bacteria, there were no significant differences among treatments; however, cellulolytic bacteria in the CM group were higher than in CC group (5.6-6.6 vs 2.3-5.4 x 10^7 CFU/g rumen content). Furthermore, total bacteria counts were similar in all treatments; meanwhile, protozoal populations were found dramatically increased as the level of cassava chip increased.

Effect on nitrogen balance and microbial nitrogen supply

As shown in Table 5, N balance in terms of N absorption and retention were similar among treatments (P>0.05). Nitrogen intakes were similar (in the range of 68.7 to 74.9 g/day) for all animals fed different diets. Furthermore, N absorbed, excreted in feces and urine in all groups were not significantly (P>0.05) affected by either energy sources or level of supplementation. Fecal-N output, urine-N excretion, N-balance of dairy steers and allantoin excretion in urine were not significantly (P<0.05) different among dietary treatments. The dairy steers were in positive N-balance. Urinary allantoin was in the range of 28.1 to 45.9 mmol/day/kg $BW^{0.75}$ for the four dietary treatments. The results on EMNS based on OMDR was significantly (P<0.05) different among energy sources and levels of supplementation. Dairy steers fed CC1 had higher EMNS (10.7 g N/kg of OMDR) than those fed CM1, CC2 and CM2 (7.7, 5.2 and 4.3 g N/kg of OMDR), respectively.

Discussion

Composition of the diet

The chemical analysis of CC, CM, CSM and UTS are presented in Table 1. The UTS had 8.7 % CP, which was slightly lower than those reported by Wanapat (1999).

Table 4. Influence of different energy levels on ruminal bacteria, protozoa, fungi population, total viable, amylolytic, proteolytic and cellulolytic bacteria in dairy steers.

Item	Treatment [d]				Contrast [e]			
	CM1	CM2	CC1	CC2	SEM	ES	L	ES x L
Rumen microbes (cells/g)								
Bacteria (x 10^{10})	7.9[a]	8.8[ab]	7.3[a]	7.5[b]	0.53	*	NS	NS
Protozoa (x 10^6)	1.0[a]	1.3[a]	1.5[b]	1.7[b]	4.87	NS	*	NS
Fungal zoospores (x 10^6)	1.1	1.0	1.1	1.0	1.56	NS	NS	NS
Viable bacteria (CFU/g)								
Total (x 10^7)	17.4	15.2	17.3	13.7	3.25	NS	NS	NS
Amylolytic (x 10^7)	10.1	8.9	11.4	10.6	2.26	NS	NS	NS
Proteolytic (x 10^6)	4.5	4.2	4.3	7.1	0.82	NS	NS	NS
Cellulolytic (x 10^7)	6.6	5.9	5.4	2.3	1.74	NS	NS	NS

[abc] **Values in the same row with different superscripts differ ($p<0.05$).**
[d] **CM1 = Corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW.**
[e] **Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = $P<0.05$, NS = $P>0.05$.**

Table 5. Nitrogen balance (g/d), excretion of purine derivatives (mmol/d) and microbial protein supply in dairy steer given different energy levels.

Item	Treatment [d]				Contrast [e]			
	CM1	CM2	CC1	CC2	SEM	ES	L	ES x L
Nitrogen balance (g/d)								
N intake	70.7	74.9	68.7	72.6	7.27	NS	NS	NS
Faecal N	2.1	3.0	2.3	3.0	0.33	NS	NS	NS
Urinary N	7.1	6.9	4.9	5.7	1.18	NS	NS	NS
N absorption	68.6	71.9	73.7	69.6	8.60	NS	NS	NS
N retention	61.5	65.0	68.9	63.9	8.13	NS	NS	NS
Purine derivative (PD) (mmol/d)								
Allantoin excretion	37.7	29.1	45.9	28.1	5.61	NS	NS	NS
Allantoin absorption	27.9	17.6	37.6	16.5	6.61	NS	NS	NS
Microbial protein supply (g N/d)	20.3	12.8	27.3	12.0	4.81	NS	NS	NS
EMNS (g N/kg of OMDR)[1]	7.7[ab]	4.3[a]	10.7[b]	5.2[ab]	1.82	NS	*	NS

[ab] **Values on the same row with different superscripts differ ($p<0.05$).**
[c] **CM1 = Corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW.**
[d] **Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = $P<0.05$, NS = $P>0.05$.**
[1] **EMNS = efficiency microbial nitrogen synthesis, OMDR = organic matter truly digested in the rumen (Chen and Gomes, 1995).**

Effect on feed intake

Intake of UTS by dairy steers in treatments with supplementation of CM and CC were not significantly different. Moreover, apparent total-tract digestibility (%) of OM and NDF were similar in all groups while digestibility of DM, CP

and ADF were significantly different in all groups. In general, rate of digestion of carbohydrates is the major factor controlling the energy available for growth of rumen microbes (Hoover and Stockes, 1991). Mertens (1977) concluded that changes in the composition of cell wall involving lignin and possibly silica limited the potential extent of digestion whereas the rate of digestion is limited by the chemical entities other than by crystalline or physical nature of fiber. It is possible that the high fibrous fraction (ADL) could have attributed to lower digestibility (Hart and Wanapat, 1992), especially a large proportion lignified cell walls with low fermentation rate and digestibility, leading to low rate of disappearance through digestion or passage and limited feed intake. In this case, cassava chip, oats, wheat and barley contain high soluble fractions of starch and sugar and can be added to diets to increase utilization of ruminal ammonia-N for microbial protein synthesis. However, previous reports (Hoover, 1986) have suggested that the reduced pH decrease digestion of fibers. Higher degradation rates can result in a substantial decrease in ruminal pH and fiber digestibility thus reducing feed intake. Moreover, when ruminal pH was reduced below 6.3 in dairy cows, ADF digestion could be reduced at 3.6 % unit per 0.1 pH and may result in depressed feed intake (Erdman, 1998). Grant (1994) has reported that source of starch influenced the rate of NDF digestion differently at pH 6.8 from 5.5 and led to dramatic differences in the apparent extent of ruminal NDF digestion using corn and sorghum starch. On the other hand, Lebzien and Engling (1995) have undertaken a comparison of cassava, corn, barley and wheat as sources of starch in non-lactating dairy cow diets. They found that the source of starch had no effect on silages and total feed intake, ruminal pH or total VFA concentration in rumen fluid. Digestibility of crude fiber was lower when barley or wheat was included in the diets of cows. In addition, they reported higher flows of starch to the duodenum in animals fed corn-starch than when fed cassava. Total tract digestibility of cassava has been reported as ranging from 98.9 to 100 percent of intake (Lebzien and Engling, 1995).

Effect on rumen fermentation

Rumen parameters of temperature, rumen pH, ammonia nitrogen (NH_3-N) and blood-urea nitrogen (BUN) concentration were measured. The temperature of the rumen was not significantly affected by treatments and all were within a normal range. Ruminal pH between CM and CC group were significantly different at 0, 1, 2, 4 and 6 h-post feeding. Especially, high level of supplementation of CC2 was the lowest in ruminal pH, while supplementation of CM1, CM2 and CC1 were similar among treatments. Normally, ruminant animals depend on cellulolytic bacteria to digest cellulose, but these bacteria cannot resist the low ruminal pH, and an increase in pH gradient leads to anion toxicity (Russell and Wilson, 1996). In addition, most ruminal bacteria prefer pH near neutrality for growth, although some species (e.g., *Steptococcus bovis* and *Prevotella ruminicola*) can grow in pH 5 to 6 ranges. The predominant ruminal cellulolytic bacteria are particularly sensitive to low pH. None of three predominant cellulolytic species grow at pH < 6.0 (Shi and Weimer, 1992; Weimer, 1993). Moreover, Weimer *et al.* (1999) also reported that populations of three prominent fiber-digesting bacteria (*Ruminococcus albus*, *Ruminococcus flavefacians* and *Fibrobacter succinogenes*) were not affected by prolonged period of ruminal pH below 6.0. Chronic decreases in ruminal pH are most easily explained by VFA, ruminal motility, and fluid dilution rate. Ruminal movements are triggered by the presence of particulate materials in the rumen, and concentrate-fed cattle do not ruminate as often as forage-fed cattle (Church, 1969). Because VFA absorption is a passive process (Ash and Dobson, 1963), the transfer of VFA from the lumen to the surface of the epithelium via rumen movements would increase the removal of VFA from the rumen.

Ruminal NH_3-N concentration was significantly affected by energy source and was lower than optional ruminal NH_3-N (15-30 mg %, Boniface *et al*., 1986; Perdok and Leng, 1990) for improving rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake. However, NH_3-N concentrations in all diets were

above the minimum level of 50 mg/L to support maximum growth rates of rumen bacteria (Satter and Slyter, 1974). Furthermore, blood urea-nitrogen concentration was significantly different among treatments. The differences in NH_3-N and BUN concentrations among treatments may have been related directly to CP levels of concentrate. Preston *et al*. (1965) reported that concentrations of BUN are highly correlated with protein intake and reflected the level of ammonia production in the rumen. This would indicate that available rumen NH_3-N could be used and/or absorbed in the rumen for further synthesis. This study revealed that incorporation of concentrate has increased NH_3-N concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria which could achieve maximum fermentation (Satter and Slyter, 1974).

Total VFA concentration was not significant by influenced dietary treatments. However, total VFA concentration in all diets was in normal concentrations of 70 to 130 mM, (France and Siddons, 1993). In addition, the molar percentage of acetate in dairy steers fed CM1 and CC1 were significantly higher than in those fed CM2 and CC2. According to Murphy *et al.* (1982), ruminal fermentation of structural carbohydrates such as cellulose and hemicellulose in diets exceeding 60% roughage would yield high proportions of acetate and butyrate, respectively. The relatively high proportion of acetate and low proportion of propionate in CM1 and CC1 as compared to CM2 and CC2 could be due to the high content of ADF and low content of hemicellulose. Furthermore, molar percentage of propionate in dairy steers fed either CM2 or CC2 were relatively higher than those fed CM1 or CC1. The changes in acetate and propionate concentrations resulted in a decrease in acetate: propionate ratio when fed CM2 or CC2 and increased C2/C3 ratio in CM1 or CC1 groups. Satter and Esdale (1968) proposed that, although acetate and propionate are the important metabolites of lactate in the rumen, acetate is usually only an intermediate and is used in the synthesis of butyrate. The oxidation of lactate to pyruvate generates two hydrogen atoms, and formation of butyrate from acetate may serve as an electron sink. Formation of propionate from lactate is an alternative way of maintaining the oxidation reduction balance but seems less favored. Satter and Esdale (1968) reported that acetate and butyrate production from lactate was pH-dependent, with acetate production maximal at higher pH and butyrate production at lower pH. The inverse relationship between acetate:propionate ratio and the amount of concentrate in the diet has often been explained by the tendency of fiber fermenting bacteria to produce acetate and starch-fermenting bacteria to produce propionate.This generalization is, however, not supported by the characteristics of pure cultures. *Ruminococcus albus* produces large amounts of acetate, but *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* produce mostly succinate, an intermediate that is ultimately converted to propionate. Some starch-fermenting bacteria can produce succinate or propionate but most are also able to produce large amounts of acetate (Hungate, 1966).

Effect on rumen microbes

Supplementation of energy sources from CM has resulted in an increase in cellulolytic, proteolytic and total viable bacteria populations whilst amylolytic and proteolytic bacteria populations were dramatically decreased. However, it should be noted that protozoal populations in all CC groups were higher than those in the CM groups. The high level of cassava-based diets had a higher number of protozoa than corn-based diets. The presence of protozoa in the rumen can also affect rumen fermentation of starch. As cellulolytic bacteria are sensitive to pH, when a high level of starch is fed, the pH may be decreased below 6.0. At this pH the cellulolytic bacteria are inhibited and feed intake depressed (Russell and Wilson, 1996). Optimum pH for maximum microbial growth is between 6.5 to 7.0 (Hungate, 1966). NH_3-N is an essential source of nitrogen for microbial protein synthesis. Furthermore, Song and Kennelly (1990) found that total mixed bacteria tended to increase with increasing level of ammonia nitrogen in the rumen fluid of cattle. The ranges of NH_3-N

level for optimal rumen ecology has been reported to be 15 to 30 mg % (Leng, 1999). In addition, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depends on the rate of soluble sugars and starches in the ration and also pH. Moreover, if the ration is based on grain, protozoa engulfment of starch grains can modulate pH and protect the animals from acidosis (Russell and Hespell, 1981). However, the decrease in protozoal count may attributed to the increase in fungal zoospores per ml rumen fluid, as removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981).

Urinary excretion of purine derivatives and microbial nitrogen supply

Excretion of allantoin in the urine was low in all diets. There was no significant difference between the amount of allantoin excreted in urine and microbial-n synthesis in the rumen. The higher microbial nitrogen supply in dairy steers fed CCL1 may be due to synchronization of the available fermentable energy and degradable nitrogen in the rumen. Moreover, the rate of digestion of carbohydrates is a major factor controlling the energy available for microbial growth (Hoover and Stokes, 1991). Furthermore, the efficiency of rumen microbial protein synthesis was significantly higher in dairy steers fed CC1 supplement. However, variability in efficiency of microbial protein synthesis exists as a result of various factors like concentration and sources of nitrogen and carbohydrates.

Conclusions

Based on this experiment it could be concluded that differences in energy sources between CM and CC and supplementation level could affect rumen ecology. Overall, cassava chip resulted in similar rumen parameters as compared to corn at high supplementation level, lowering ruminal pH. Therefore, further studies of high level of cassava chip in concentrate should be conducted particularly with non-protein nitrogen (NPN).

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References

AOAC. 1985. Official Methods of Analysis. Association of Official Analysis Chemists, DC.

ARC. 1990. The Nutrient Requirements of Ruminant Livestock. Suppl.1.Commonwealth Agricultural Bureaux, Slough, Farnham Royal, UK.

Ash, R.W. and Dobson, A. 1963. The effect of absorption on the acidity of ruminal contents. J. Physiol. 169: 39-61.

Boniface, A.N., Murrah, R.M. and Hogan, P. 1986. Optimum level of ammonia in the rumen liquid of cattle fed tropical pasture hay. **In**: The Proc. Aust. Soc. Anim. Proc. 1: 151-154.

Chen, X.B. and Gomes, M.J. 1995. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives an Overview of the Technical Details. International Feed Resources Unit, Rowel Research Institute, Aberdeen, UK.

Chen, X.B., Kyle, D.J. and Ørskov, E.R. 1993. Measurement of allantoin in urine and plasma by high-performance liquid chromatography with pre-column derivatization. J. Chromatogra. 617: 241-247.

Church, D.C. 1969. Digestive physiology and nutrition of ruminants. **In**: Digestive Physiology Vol. 1.O.S.U. Book Stores, Inc., Corvallis, OR.

Crocker, C.L. 1967. Rapid determination of urea nitrogen in serum or plasma without deproteinzation. Am. J. Med. Techn. 33: 361-365.

Demeyer, D.I. 1981. Rumen microbes and digestion of plant cell walls. Agric. Environ. 6: 295-337.

Erdman, A. 1998. Dietary buffering requirements of the lactating dairy cows. J. Dairy Sci. 71: 3246-3266.

France, J. and Siddons, R.C. 1993. Volatile fatty acid production. **In**: Quantitative Aspects Ruminant Digestion and Metabolisim (Eds., J. M. Forbes and J Frace). C.A.B. International, Willingford, UK.

Galyean, M. 1989. Laboratory Procedure in Animal Nutrition Research. Department of Animal and Life Science. New Mexico state University, USA.

Goering, H.K. and Van Soest, P.J. 1970. Forage Fiber Analysis (apparatus, reagent, procedures and some application). Agric. Handbook No. 379, ARS, USDA, Washington, D.C.

Grant, R.J. 1994. Influence of corn and sorghum starch on the *in-vitro* kinetics of forage fiber digestion. J. Dairy Sci. 77: 1563-1569.

Hart, F.J. and Wanapat, M. 1992. Physiology of digestion of urea-treated rice straw in swamp buffalo. Asian-Aust. J. Anim. Sci. 5: 617-622.

Holzer, Z., Aharoni, Y., Lubimov, V. and Brosh, A. 1997. The feasibility of replacement of grain by tapioca in diets for growing-fatting cattle. Anim. Feed Sci. Technol. 64: 133-141.

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69: 2755-2766.

Hoover, W.H. and Stokes, S.R. 1991. Balancing carbohydrate and proteins for optimum rumen microbial yield. J. Dairy Sci. 74: 3640-3644.

Hungate, R.E. 1966. The Rumen and It's Microbe. Academic Press, New York, NY.

Hungate, R.E. 1969. A Roll Tube Method for Cultivation of Stric Anaerobes. **In**: Methods in Microbiology. (Eds., J. R. Norris and D. W. Ribbons). New York.

IAEA. 1997. Estimation of The Rumen Microbial Protein Production from Purine Derivatives in Rumen. Animal production and health section. Vienna, Austria, p. 49.

Jouaney, J.P. and Ushida, K. 1999. The role of protozoa in feed digestion. Asian-Aust. J. Anim. Sci. 12: 113-126.

Kanjanaputhipong, J., Bautoug, N., Kanto, U., Juttupornpong, S. and Chaw-uthai, W. 2001. Cassava chips and ground corn as sources of total non-fiber carbohydrate in total mixed ration for dairy cows. Asian-Aust. J. Anim. Sci. 14: 206-210.

Lebzien, P. and Engling, P. 1995. On the influence of 2 rations of grass-silage and concentrate mixtures with different sources of carbohydrates on rumen fermentation and nutrient digestibility in dairy-cows. J. Anim. Physiol. 74: 208-218.

Leng, R.A. 1999. Feeding strategies for improving milk production. **In**: Smallholder Dairying in the Tropics. (Eds., L. Falvey and C. Chantalakana). International livestock Research Institute (ILRI), Nairobi, Kenya.

Mertens, D.R. 1977. Dietary fiber components: relationship to the rate and extent of ruminal digestion. Proc, Fed. Am. Sci. Exp. Biol. 36: 187-192.

Murphy, M.R., Baldwin, L.R. and Kung, L.J. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55: 411-421.

Perdok, H.B. and Leng, R.A. 1990. Effect of supplementation with protein meal on the growth of cattle given a basal diet of untreated or ammoniated rice straw. Asian-Aust. J. Anim. Sci. 3: 269-279.

Preston, R.L., Schnakanberg, D.D. and Pander, W.H. 1965. Protein utilization in ruminants. I. Blood urea nitrogen as affected by protein intake. J. Nutr. 86: 281-287.

Russell, J.R. and Hespell, R.B. 1981. Microbial rumen fermentation. J. Dairy Sci. 64: 1153-1169.

Russell, J.B. and Wilson, D.B. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH. J. Dairy. Sci. 79: 1503-1511.

SAS. 1998. SAS/STAT User's Guide. Version 6.12. SAS Inst. Inc., Cary, NC.

Satter, L.D. and Esdale, W.J. 1968. *In vitro* lactate metabolism by ruminal ingesta. Appl. Microbiol. 16: 680-688.

Satter, L.D. and Slyter, L.L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr. 32: 199-208.

Shi, Y. and Weimer, P.J. 1992. Response surface analysis of the effects of pH and dilution rate on ruminococcus flavefaciens FD-1 in cellulose-fed continuous culture. Appl. Environ. Microbiol. 58: 2583-2589.

Snedecor, G.W. and W.C. Cochran. 1967. Statistical Methods. 6th Edition. The Iowa State University Press, Ames, IA.

Song, M.K. and Kennelly, J.J. 1990. Ruminal fermentation pattern, bacteria population and ruminal degradation of feed ingredients as influenced by ruminal ammonia concentration. J. Anim. Sci. 68: 1110-1120.

Steel, R.D. and Torrie, H. 1980. Principles and Procedure of Statistics. McGraw-Hill Publishing Co, New York.

Wanapat, M. 1999. Feeding of Ruminants in The Tropicals Base on Local Feed Resources. Khon Kaen Publising Company Ltd., Khon Kaen, Thailand.

Wanapat, M. 2000. Rumen manipulation to increase the efficient use of local feed resources and productivity of ruminants in the tropics. Asian-Aust. J. Anim. Sci. 13: 59-67.

Wanapat, M. 2003. Manipulation of cassava cultivation and utilization to improve protein to energy biomass for livestock feeding in the tropics. Asian-Aust. J. Anim. Sci. 16: 463-472.

Weimer, P.J. 1993. Effect of dilution rate and pH on the ruminal cellulolytic bacteria *Fibrobacter succinogenes* S85 in cellulose-fed continuous culture. Appl. Microbiol. 160: 288-295.

Weimer, P.J., Waghorn, G.C., Otd, V.L. and Mertens, D.R. 1999. Effect of diet on population of three species of ruminal cellulolytic bacteria in lactating dairy cows. J. Dairy Sci. 82: 122-134.

Zinn, A.R. and Owens, F.N. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66: 157-163.